ABSTRACT: Metabolic demand for sulfur-containing AA increases during inflammation in nonruminants. Therefore, Met supplementation may alleviate the negative effects of infection on N balance. Effects of gram-negative bacterial lipopolysaccharide (LPS) and supplemental dietary Met on N balance, serum hormones and haptoglobin, and plasma urea-N and AA were evaluated in 20 Angus-cross steers (BW = 262 ± 6.3 kg). Treatments (2 × 2 factorial) were infusion of no LPS (−LPS) or a prolonged low dose of LPS (+LPS) and dietary supplementation of no (−MET) or 14 g/d (+MET) of rumen-protected Met (providing 7.9 g/d of dl-Met). Steers were adapted to a roughage-based diet (DMI = 1.4% of BW daily) and supplemental Met for 14 d, and were then infused (1 mL/min via intravenous catheter) with LPS on d 1 (2 µg/kg of BW) and 3 (1 µg/kg of BW) of a 5-d collection period. Blood was collected on d 1, before LPS infusion, and at 2, 4, 6, 8, 10, 12, and 24 h after LPS challenge. Diet samples, feed refusals, feces, and urine were collected daily for 5 d. Rectal temperature and serum concentrations of cortisol, prolactin, tumor necrosis factor-α, and haptoglobin increased, whereas thyroxine and triiodothyronine decreased for +LPS vs. −LPS steers (LPS × h; \( P < 0.01 \)). Plasma urea-N was greater for +LPS than −LPS steers (LPS; \( P = 0.03 \)), and serum IGF-1 was not affected (\( P ≥ 0.26 \)) by LPS or Met. Plasma concentrations of Thr, Lys, Leu, Ile, Phe, Trp, Asn, Glu, and Orn decreased, plasma Ala increased, and Gly and Ser initially increased, then declined in +LPS vs. −LPS steers (LPS × h; \( P ≤ 0.04 \)). Plasma Met was greater for +MET than −MET steers before LPS infusion, but declined in +MET steers after LPS infusion (LPS × Met × h; \( P < 0.01 \)). By design, DMI was not different, but DM digested was less (\( P = 0.04 \)) for +LPS than −LPS steers. Infusion of LPS did not affect (\( P ≥ 0.24 \)) N intake, fecal N excretion, or N digested, but resulted in greater (\( P = 0.01 \)) urinary N excretion and less (\( P < 0.01 \)) N retention. The absence of an LPS × Met interaction (\( P = 0.26 \)) for N retention indicates that supplemental Met does not improve the N utilization of growing beef steers exposed to a gram-negative bacterial endotoxin. Decreases in plasma concentrations of several essential AA in +LPS steers suggest that metabolic demand for these AA likely increased in steers exposed to endotoxin.

Key words: endotoxin challenge, methionine, steer

INTRODUCTION

Clinical and metabolic alterations associated with the inflammatory response to gram-negative bacterial pathogens occur because of recognition of the lipopolysaccharide (LPS) component of bacterial cell walls (Cullor, 1992). This inflammatory response can be induced experimentally by administration of purified LPS (Steiger et al., 1999).

Cole et al. (1986) and Orr et al. (1988) reported that inflammation increased urinary N excretion and decreased N retention in cattle. Reeds and Jahoor (2001) speculated that the increased N excretion observed in humans during sepsis occurred because of imbalances between the supply of AA derived from tissue protein...
and the AA composition of acute-phase proteins. Insufficient supplies of sulfur-containing AA may limit hepatic acute-phase protein production (Grumble and Grimble, 1998), and plasma concentrations of Met in sheep have been shown to decrease in response to endotoxin (Hofford et al., 1996). Sulfur-containing AA are preferentially retained during the inflammatory response in humans (Santangelo, 2002), and Malmezat et al. (1998) reported an 80% increase in the transsulfuration of Met to Cys during sepsis in rats. Furthermore, supplementation of Met increased total lymphocyte numbers in humans (Van Brummelen and du Toit, 2007) and enhanced lymphocyte proliferation in dairy cattle (Soder and Holden, 1999). Laurichesse et al. (1998) identified Met as a limiting AA in humans with the acquired immunodeficiency syndrome.

Considering the role of Met in AA metabolism during sepsis in monogastric species, and that Met is a limiting AA in growing steers fed diets low in ruminally undegradable protein (Greenwood and Titgemeyer, 2000), we hypothesized that supplementation of Met to growing beef steers would alleviate the negative effects of infection on N balance. Therefore, the objective was to evaluate the effects of supplemental dietary Met on N balance, serum hormones, and plasma AA of growing beef steers exposed to gram-negative bacterial LPS.

**MATERIALS AND METHODS**

Procedures for this study were approved by the New Mexico State University Institutional Animal Care and Use Committee.

**Animals, Diets, and Treatments**

Twenty Angus-cross steers (262 ± 6.3 kg of initial BW) in a randomized block design were individually maintained in tie stalls of a metabolism building with continuous lighting and evaporative cooling (21 ± 1.8°C). Tie stalls were equipped with rubber mats (2.54 cm thick) and automatic water troughs (Nelson Manufacturing, Cedar Rapids, IA). Steers were allowed free access to fresh water and were limit-fed a roughage-based diet (Table 1) at approximately 1.4% of BW daily (DM basis) in equal portions at 0700 and 1900 h. This amount of intake was selected to minimize intake differences among treatments and to correspond with dietary intakes of stressed, newly received feedlot calves (NRC, 2000). The experimental period was 19 d, which provided 14 d for adaptation to facilities, diets, and dietary treatments, followed by a 5-d collection period.

Treatments, arranged as a 2 × 2 factorial, were infusion of no LPS (−LPS) or a prolonged low dose of LPS (+LPS; Steiger et al., 1999), and dietary supplementation of no (−MET) or 14 g/d (+MET) of rumen-protected Met (Smartamine M, Adisseo, Alpharetta, GA). Based on product specifications, the 14 g/d of rumen-protected Met (≥70% DL-Met, ≥90% protection in the rumen, and ≥90% release in the abomasum) supplied a minimum of 7.9 g/d of absorbable DL-Met. Rumen-protected Met was divided into 2 equal portions, and 7 g was thoroughly mixed with the diet before each feeding at 0700 and 1900 h. Administration of LPS was facilitated through an indwelling jugular catheter (J-457A, Jorgensen Laboratories, Loveland, CO) inserted on d 14 of the experiment. The LPS (Escherichia coli O55:B55, Sigma Chemical Co., St. Louis, MO) solution was prepared by dissolving the individual LPS dose of each steer (2 µg/kg of BW) in 100 mL of sterile saline immediately before infusion. The LPS was infused at 1 mL/min by using an electronic syringe pump (Model 230, KD Scientific Inc., Holliston, MA) at 3 h after feeding on the first day of the collection period (d 15 of the experiment). This procedure was repeated on the third day of the collection period (d 17); however, the dose of LPS was reduced to 1 µg/kg of BW in 50 mL of sterile saline because 1 Met-supplemented steer died at approximately 10 h after the first dose of LPS. For −LPS steers, an equal volume of sterile saline was administered at a rate similar to +LPS steers.

**Table 1. Diet composition (DM basis)**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %</td>
<td></td>
</tr>
<tr>
<td>Cracked corn grain</td>
<td>35.0</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>25.0</td>
</tr>
<tr>
<td>Corn silage</td>
<td>20.0</td>
</tr>
<tr>
<td>Sorghum, sudan hay</td>
<td>12.4</td>
</tr>
<tr>
<td>Molasses</td>
<td>4.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>1.2</td>
</tr>
<tr>
<td>Casein</td>
<td>1.0</td>
</tr>
<tr>
<td>Urea</td>
<td>0.61</td>
</tr>
<tr>
<td>Minerals&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.73</td>
</tr>
<tr>
<td>Vitamins&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.03</td>
</tr>
<tr>
<td>Rumensin-80&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>1</sup>Supplied (% of total dietary DM): salt (0.30), limestone (0.30), di-calcium phosphate (0.11), zinc sulfate (0.009), copper sulfate (0.004), sodium selenite (0.000009).

<sup>2</sup>Supplied 1,500 IU of vitamin A and 100 IU of vitamin E per kilogram of dietary DM.

<sup>3</sup>Supplied 33 mg of monensin per kilogram of dietary DM (Elanco Animal Health, Indianapolis, IN).

<sup>4</sup>ME, Mcal/kg = (4.103 − 0.0446 × %ADF) × 0.82 (Harlan et al., 1991).

<sup>5</sup>NEm, Mcal/kg = 1.37 × ME − 0.138 × ME<sup>2</sup> + 0.0105 × ME<sup>3</sup> − 1.12 (NRC, 2000).

<sup>6</sup>NEm, Mcal/kg = 1.42 × ME − 0.174 × ME<sup>2</sup> + 0.0122 × ME<sup>3</sup> − 1.65 (NRC, 2000).
Collections

For determination of passage rates, all steers were fed 400 g of Cr-EDTA and 120 g of Yb-labeled diet before the 0700 h feeding on d 15. The Yb-labeled diet was prepared by soaking 120 g of the basal diet (Table 1) in 600 mL of a 0.3% YbCl₃ (wt/vol) solution (Stock No. 42816, Alfa Aesar, Ward Hill, MA) for 48 h (stirred every 12 h). This mixture was strained (2-mm fiberglass screen), washed (with tap water every hour for 6 h to remove unbound Yb), then dried at 55°C for 36 h in a forced-air oven (Model POM-326F, Blue M Electric Company, Blue Island, IL). A portion of the basal diet (400 g) was also mixed with 80 mL of a Cr-EDTA solution (3.96 g of Cr/L; prepared according to Binnerts et al., 1968) and dried at 55°C in a forced-air oven for 36 h. After feeding the markers to steers, samples of feces were retrieved from the rectum every 24 h for 5 d and frozen for later analysis of Cr and Yb.

Diet samples were collected on d 14 through 18 to correspond with the collection of total feed refusals (if any), and fecal and urinary excreta were collected on d 15 to 19. Feces were collected into fecal bags and urine was collected via vacuum pouches into 20-L vessels (No. C14907, Nasco, Modesto, CA) containing 600 mL of 3 M HCl (to minimize NH₃ loss). Total fecal and urinary output was weighed, and representative samples of feces (10%) and urine (1%) were frozen for later analysis.

On the first day of LPS challenge, rectal temperatures were measured (Cooper TM999A digital thermometer, Cooper Atkins Corp., Middlefield, CT) before LPS infusion and every 2 h for 12 h thereafter. Similarly, only on the first day of LPS challenge, blood samples (±8 mL) were collected via jugular catheters into vacuum tubes (Corvac serum separator and Monoject 15% EDTA, Kendall, Ontario, CA) before LPS infusion and at 2, 4, 6, 8, 10, 12, and 24 h after infusion of LPS. Blood samples obtained for the collection of serum were allowed to coagulate at room temperature for 30 min, whereas samples obtained for plasma were immediately placed on ice. All blood samples were centrifuged (Sorvall RT600B, Thermo Electron Corp., Asheville, NC) at 1,500 × g for 20 min at 10°C. Serum and plasma samples were immediately decanted with transfer pipettes into 7-mL vials and frozen for later analysis.

Sample Analysis

Diet, feed refusals, and fecal samples were dried at 55°C in a forced-air oven (Model POM-326F, Blue M Electric Company) and ground to pass a 2-mm screen in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ). Ground samples were analyzed for DM (105°C for 24 h) in a convection oven (Model 845, Precision Scientific Group, Chicago, IL). The N concentrations of feed, feces, and urine samples were determined by total combustion (Leco FP-528, Leco Corp., St. Joseph, MI) to calculate N retention. Fecal grab samples were digested by microwave assistance (method 3015A; US EPA, 2007) before analysis for Cr and Yb via inductively coupled plasma spectrometry (Optima 4300, Perkin Elmer, Wellesley, MA). Liquid (Cr) and solid (Yb) passage rates (%/h) were determined from the slope of the natural log of the fecal concentrations of Cr (24 to 96 h after feeding) and of Yb (48 to 120 h after feeding) regressed against hour (Grosvum and Williams, 1973).

Urea-N concentrations of plasma (0, 2, 4, 6, 8, 10, and 12 h after LPS infusion) and urine were determined colorimetrically. Plasma and urine samples (4 µL) plus 200 µL of urease reagent (Infinity TR12421, Thermo Scientific, Waltham, MA) in 96-well plates were placed on a shaker for 15 s, then incubated at 37°C for 5 min. The 96-well plates were exposed to 4°C for 5 min and then read at 340 nm on a microplate reader (Biotek ELx808, Biotek Instruments Inc., Winooski, VT). Ammonia-N concentrations of urine were determined colorimetrically according to the procedure of Broderick and Kang (1980).

Serum concentrations of cortisol, triiodothyronine (T₃), and thyroxine (T₄) were determined in duplicate by solid-phase RIA, using components of commercial kits (Siemens Diagnostic, Los Angeles, CA). These kits use antibody-coated tube technology, and assays were performed without prior extraction of the individual hormones from serum. The cortisol, T₃, and T₄ assays were validated in ruminant serum as described by Kimya et al. (2004), Wells et al. (2003), and Richards et al. (1999), respectively, and within- and between-assay CV were less than 7%. Serum IGF-1 and prolactin concentrations were determined in duplicate by double-antibody RIA as described by Berrie et al. (1995) and Spoon and Hallford (1989), respectively, using primary antisera and purified standard and iodination preparations supplied by the National Hormone and Peptide Program (Harbor-UCLA Medical Center, Torrance, CA). Assay of total serum IGF-1 was conducted after acid-ethanol inactivation of binding proteins (intra- and interassay CV of 4 and 6%, respectively, and sensitivity of 4 ng/mL). Serum prolactin was quantified in a single assay with a CV of 9% and sensitivity of 4 ng/mL. Serum samples obtained at 0, 2, 4, 6, 8, 10, and 12 h after LPS infusion were used for determination of cortisol and prolactin concentrations, and serum samples collected at 0, 4, 8, and 12 h after LPS infusion were used for determination of IGF-1, T₃, and T₄ concentrations.

Concentrations of tumor necrosis factor-α (TNFα) were quantified in serum samples obtained at 0, 2, 4, and 6 h after LPS infusion by RIA, using primary antisera obtained from AbD Serotec (Kidlington, Oxford, UK; rabbit antihemovin TNFα). For use in the assay, the anti-TNFα was diluted 1:2,000 in PBS-EDTA plus 1:400 normal rabbit serum. Recombinant bovine TNFα (R&D Systems, Minneapolis, MN) was used as the standard and to prepare the ¹²⁵I-TNFα (chloramine T-sodium metabisulfite method). Stock standard solu-
tions containing 10 and 100 ng/mL of TNFα were used to prepare the standard curve containing 0, 0.25, 0.50, 1, 2, 4, and 8 ng/tube. Standard tubes were normalized to 0.15 mL with PBS + 1% BSA, after which each tube received 0.1 mL of low-TNFα bovine serum to adjust standards for possible matrix interference. The assay was conducted in 12 × 75 mm polypropylene culture tubes to which 0.1 mL of steer serum and 0.15 mL of PBS + 1% BSA were added. Each tube then received 0.2 mL of anti-TNFα and 0.1 mL of 125I-TNFα (approximately 30,000 counts/min per tube in PBS + 1% BSA). All tubes were vortexed briefly and incubated overnight at 4°C. On the second day, sheep anti-rabbit gamma globulin (0.2 mL in PBS-EDTA) was added and tubes were incubated overnight at 4°C. On the third day, tubes were centrifuged (2,500 × g at 4°C), the supernatant was discarded, and the precipitate was counted in a gamma counter. Concentrations of TNFα were computed by using the 4-parameter logistic method. Addition of increasing amounts of bovine serum resulted in TNFα concentrations that paralleled the standard curve. The detection limit of the assay was 0.7 ng/tube. Specific binding of 125I-TNFα to the antibody averaged 30%, and addition of 0.25 ng of TNFα resulted in displacement of 7% of 125I-TNFα from the body averaged 30%, and addition of 0.25 ng of TNFα to the anti-standard curve. The detection limit of the assay was resulted in TNFα concentrations that paralleled the standard curve. The detection limit of the assay was 0.7 ng/tube. Specific binding of 125I-TNFα to the antibody averaged 30%, and addition of 0.25 ng of TNFα resulted in displacement of 7% of 125I-TNFα from the antibody. When 1, 5, or 10 ng of TNFα was added to bovine serum, 112, 105, or 93% was recovered, respectively. Study samples were quantified in 2 assays with intra- and inter-assay CV of 12 and 16%, respectively.

Serum samples obtained before LPS infusion and 2, 4, 6, 8, 10, 12, and 24 h thereafter were submitted to the Kansas State University Veterinary Diagnostic Laboratory (Manhattan, KS) for analysis of haptoglobin as described by Smith et al. (1998). Concentrations of AA were determined in plasma samples obtained before LPS infusion and at 2 through 12 h after administration of LPS via GLC by using a commercially available kit (EZ:FAAST No. KGO-7165, Phenomenex, Torrance, CA). The kit supplied all necessary reagents for solid-phase extraction and derivatization. Amino acids were extracted from 100 µL of plasma and eluted, the AA were derivatized, and the eluant was allowed to separate into 2 layers. An aliquot of the organic layer was collected into glass scintillation vials and analyzed for AA on a gas chromatograph (CP-3800, Varian, Walnut Creek, CA) by using a split-injection protocol (2 µL of sample at 250°C) with helium (1.5 mL/min) as the carrier gas. Intra- and interassay CV for serum haptoglobin and plasma AA were less than 15%.

**Statistical Analysis**

All data were analyzed statistically as a randomized block design by using the MIXED procedure (SAS Inst. Inc., Cary, NC). Because of a limited number of tie stalls (12) in the metabolism facility, data collection occurred over 2 periods. Therefore, the experiment was blocked (12 steers in block 1, and 8 steers in block 2) by collection date, with steer as the experimental unit. One Met-supplemented steer that received +LPS died at approximately 10 h after infusion, and all data collected from this steer were excluded from the statistical analysis.

For dependent variables without repeated observations (dietary intake, digestibility, N balance, and passage rates), the model included the effects of LPS, Met, and the LPS × Met interaction, and block was included as a random effect. Rectal temperature, serum cortisol, prolactin, IGF-1, T₃, T₄, TNFα, haptoglobin, plasma urea-N, and AA were analyzed as repeated measures (covariance structure = autoregressive order one). The sources of variation in the repeated measures analysis were block, steer, LPS, and hour, with the block and steer within treatment combination (LPS × Met) as random effects. The model included all possible interactions of LPS, Met, and hour. Data are presented as least squares means, and differences were considered significant at *P* < 0.05.

**RESULTS**

An LPS × h interaction (*P* < 0.01) was observed for rectal temperatures and serum concentrations of cortisol, prolactin, TNFα, T₃, T₄, and haptoglobin. Rectal temperatures (Figure 1) increased from 0 to 2 h in +LPS steers and were greater for +LPS than −LPS steers from 2 to 8 h, then declined and were not different from −LPS at 10 and 12 h after LPS infusion. Serum cortisol concentrations (Figure 2) increased 2 h after LPS administration and remained elevated throughout the sampling period in +LPS steers compared with −LPS steers. Serum concentrations of prolactin (Figure 2) and TNFα (Figure 3) increased in +LPS steers and were greater from 2 to 4 h after administration of LPS, but declined and were not different from those of −LPS steers at 6 h. The thyroid hormones (T₃ and T₄; Figure 4) declined in +LPS steers after infusion and remained less than in −LPS steers throughout the 12-h sampling period. Serum haptoglobin concentrations (Figure 5) were not different among +LPS and −LPS steers from 0 through 10 h after LPS infusion, but increased in +LPS steers and were greater than −LPS steers at 12 and 24 h after the LPS challenge. Serum IGF-1 (Table 2) was not affected by LPS or Met supplementation (*P* ≥ 0.26), and plasma urea-N concentrations were greater (*P* = 0.03) for LPS-challenged steers than for −LPS steers.

An LPS × Met × h interaction (*P* < 0.01) was observed for plasma Met concentrations (Figure 6). Plasma Met was greater for +MET than −MET steers before LPS infusion, but declined for +MET steers exposed to +LPS such that their plasma Met concentrations were not different from −MET steers from 4 to 12 h after LPS infusion.

An LPS × h interaction (*P* < 0.01) occurred for plasma concentrations of Thr, Lys, Leu, Ile, Phe, Trp,
Ser, Gly, Ala, Asn, Glu, and Orn. Plasma Thr concentrations (Figure 7) were greater for +LPS than −LPS steers at 0 and 2 h, and declined 4 h after infusion of LPS (LPS × h; \( P < 0.01 \)). Plasma concentrations of Lys (Figure 7) were greater in LPS-challenged steers 2 h after infusion of LPS, but declined at 4 h and were not different from −LPS steers thereafter (LPS × h; \( P < 0.01 \)). Plasma concentrations of Leu, Ile, Phe, and Trp (Figure 7) decreased from 2 to 4 h after steers were exposed to LPS and remained less than in −LPS steers throughout the 12-h sampling period, except for Leu at 6 h after LPS (LPS × h; \( P < 0.01 \)). Plasma Gly and Ser concentrations (Figure 8) were greater for LPS-challenged steers at 2 h, but declined from 2 to 4 h and remained less than −LPS steers from 6 to 12 h (LPS × h; \( P < 0.01 \)). Plasma Ala concentrations (Figure 8) increased from 0 to 2 h in response to LPS, were greater for +LPS than −LPS steers at 6 h, and tended to be greater at 4, 10, and 12 h after LPS administration. Plasma Asn concentrations (Figure 8) declined from 0 to 4 h in response to LPS and were less in +LPS than in −LPS steers from 4 to 12 h after LPS infusion (LPS × h; \( P < 0.01 \)). Plasma Glu (Figure 8) concentrations were initially greater for +LPS than −LPS steers before LPS infusion, but declined from 2 to 4 h after LPS infusion and were not different from −LPS steers thereafter (LPS × h; \( P = 0.02 \)). Plasma Orn (Figure 8) tended to be less for +LPS than −LPS steers at 4 h and were less in +LPS steers at 10 and 12 h after LPS infusion (LPS × h; \( P = 0.04 \)).

Administration of LPS decreased (\( P < 0.01 \)) plasma concentrations of Tyr, and dietary Met supplementation decreased (\( P = 0.02 \)) plasma concentrations of Val (Table 2). Plasma His, Cys, Gln, and Asp concentrations were not affected by LPS or Met (Table 2).

No LPS × Met interactions (\( P \geq 0.26 \)) were observed for dietary DM and N intakes, fecal and urinary N excretion, or passage rates (Table 3). However, DM digested was less (\( P = 0.04 \)) and dietary DMI tended (\( P = 0.13 \)) to be less for +LPS than −LPS steers. Infusion of LPS did not affect N intake (\( P = 0.37 \)), fecal N excretion (\( P = 0.82 \)), or N digested (\( P = 0.24 \)), but did result in greater (\( P = 0.01 \)) urinary N excretion and less (\( P < 0.01 \)) N retention, with +LPS steers being in a negative N balance. Supplementation of Met did not affect N intake (\( P = 0.86 \)), urinary N excretion (\( P = 0.88 \)), N digested (\( P = 0.56 \)), or N retained (\( P = 0.61 \)) despite greater fecal N excretion (\( P = 0.03 \)) for +Met than −Met steers. Liquid (Cr) and solid (Yb) passage rates were less (\( P < 0.01 \)) for steers exposed to LPS,
and were greater \((P \leq 0.03)\) in steers receiving Met supplementation.

**DISCUSSION**

**LPS Infusion**

Increases in rectal temperature; increases in serum concentrations of cortisol, prolactin, TNFα, and haptoglobin; and decreases in the thyroid hormones indicate that LPS elicited an acute stress response and stimulated hepatic acute-phase protein synthesis. Steiger et al. (1999) and Waldron et al. (2003) observed similar increases in rectal temperature, cortisol, and TNFα concentrations in response to intravenous infusion of LPS in cattle. Although prolactin is traditionally viewed as a somatolactogenic hormone, it also stimulates lymphocyte proliferation and is an essential component of the immune response (Arkins et al., 1993). Alterations in serum concentrations of cortisol, prolactin, and TNFα occur as a result of LPS-mediated stimulation of monocytes, macrophages, and endothelial cells (Tizard, 2004), and of subsequent activation of the hypothalamic-pituitary-adrenal axis by cytokines released from these immune cells (Sapolsky et al., 2000). Hepatic production of the acute-phase proteins, haptoglobin, fibrinogen, serum amyloid A, and α1-acid glycoprotein, is stimulated by cytokines (primarily IL-1, IL-6, and TNFα) in response to both experimentally induced and naturally acquired inflammation in cattle (Petersen et al., 2004). The decline in T3 and T4 concentrations observed after LPS occurs because of the development of a hypothyroid condition characterized by low T3 and T4 concentrations and reduced hepatic production of the 5′-deiodinase enzyme responsible for the conversion of T4 to T3 (Kahl et al., 2000). Cole et al. (1994) observed reduced serum T3 concentrations in cattle in response to an infectious bovine rhinotracheitis virus (IBRV) challenge. Concentrations of IGF-1 have previously been reported to decrease in response to administration of LPS in cattle (Elsasser et al., 1995), sheep (Briard et al., 2000), and rodents (Soto et al., 1998). The decline in IGF-1 concentration attributed to LPS in these species likely occurs because of reduced tissue (i.e., skeletal muscle and liver) production of IGF-1 under conditions in which the immune system is stimulated (Spurlock, 1997).

Decreases in plasma concentrations of Met, Lys, Leu, Ile, Phe, Trp, Gly, Ser, Asn, Ghu, and Orn in response to LPS reflect alterations in AA metabolism, likely caused by an increase in demand for AA by the immune system.
During inflammation, AA are required for the synthesis of acute-phase proteins, glucose precursors, plasma proteins, antibodies, free-radical scavengers, metabolic cofactors, and hormones (Li et al., 2007). Acute-phase proteins in humans contain high amounts of Phe, Trp, Lys, Cys, and Ser relative to skeletal muscle (Reeds and Jahoor, 2001) and human lymphocytes preferentially utilize the branched-chain AA during inflammation as substrates for energy or protein (i.e., antibody) synthesis (Calder, 2006). Furthermore, dietary restriction of the branched-chain AA impaired lymphocyte proliferation and increased mortality in rodents after bacterial infection (Calder, 2006). The production of glutathione, a free-radical scavenger derived from Glu, requires Cys, a product of Met transsulfuration, and Gly, a product of Ser metabolism (Wu et al., 2004). Metabolic demand for Orn may increase during sepsis because of glucocorticoid-induced upregulation of the expression of enzymes in the urea cycle (Morris, 2002). However, the observed reduction in plasma Orn may also reflect limitations in the metabolic supply of Arg, because Orn concentrations are reflective of plasma Arg concentrations (Maltby et al., 2005) and plasma Arg reportedly declines after sepsis in humans (Li et al., 2007). Arginine is an intermediate in the urea cycle and is a substrate for nitric oxide production (Calder and Yaqoob, 2004) by macrophages and monocytes in response to bacterial LPS (Mahoney and Albina, 2004). Because of the analytical procedures used, plasma Arg could not be evaluated in this study.

The observed decline in Met after LPS administration may be due to an increase in Met transsulfuration, because metabolic demand for Cys and S-adenosylmethionine increases during inflammation (Li et al., 2007). Malmezat et al. (2000) observed an 80% increase in the transsulfuration of Met to Cys in septic rats. This

### Table 2. Serum IGF-1 and plasma urea-N, His, Val, Cys, Asp, Gln, and Tyr concentrations in growing beef steers in response to Met supplementation and endotoxin (lipopolysaccharide; LPS) challenge

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment¹</th>
<th>−LPS</th>
<th>+LPS</th>
<th>SEM²</th>
<th>LPS × Met</th>
<th>LPS</th>
<th>Met</th>
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<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>5</td>
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<td></td>
</tr>
<tr>
<td>Serum IGF-1, ng/mL</td>
<td>184 192 23.5</td>
<td>182 192 23.5</td>
<td>142</td>
<td>4.3</td>
<td>0.30 0.26 0.50</td>
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<td></td>
</tr>
<tr>
<td>Plasma urea-N, mg/dL</td>
<td>8.9 9.1 1.4</td>
<td>11.6 10.3 1.4</td>
<td>9.1</td>
<td>0.36</td>
<td>0.03 0.52</td>
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<tr>
<td>Plasma AA, µM</td>
<td>36 32 7.0</td>
<td>36 31 7.0</td>
<td>32</td>
<td>1.9</td>
<td>0.56 0.60 0.68</td>
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<tr>
<td>His</td>
<td>188 14.4 7.0</td>
<td>188 14.4 7.0</td>
<td>192</td>
<td>1.11</td>
<td>0.62 0.20</td>
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</tr>
<tr>
<td>Val</td>
<td>34.7 10.6</td>
<td>34.7 10.6</td>
<td>32</td>
<td>0.99</td>
<td>0.21 0.65</td>
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<tr>
<td>Cys</td>
<td>14.7 10.6</td>
<td>14.7 10.6</td>
<td>14.4</td>
<td>0.99</td>
<td>0.21 0.65</td>
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<td></td>
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<tr>
<td>Asp</td>
<td>14.7 10.6</td>
<td>14.7 10.6</td>
<td>14.4</td>
<td>0.99</td>
<td>0.21 0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln</td>
<td>293 311 3.9</td>
<td>293 311 3.9</td>
<td>262</td>
<td>3.9</td>
<td>&lt;0.01 0.94</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Treatments were a 2 × 2 factorial arrangement of 1) no LPS (−LPS; 100 mL of sterile saline) or 2) a prolonged low dose of LPS (+LPS; 2 µg of LPS × kg of BW/100 mL of sterile saline) and dietary supplementation of 1) 0 g/d (−MET) or 2) 14 g/d (+MET) of rumen-protected Met.

²Effects of LPS × Met × h (P = 0.33 to 0.99), LPS × h (P = 0.27 to 0.99), Met × h (P = 0.26 to 0.95).

³For n = 5.

![Figure 5](image-url)
increase in Met transsulfuration was attributed to an increased metabolic demand for Cys to support sepsis-induced increases in glutathione turnover. Cysteine is also required for the production of acute-phase proteins, and S-adenosylmethionine may stimulate the production of cytokines involved in the immune response (Grimble and Grimble, 1998). Additionally, the preferential retention of sulfur coupled with the increased demand for glutathione observed during inflammation suggests that requirements for sulfur-containing AA increase during inflammation (Grimble, 2006).

Reduced passage rates attributed to LPS in this study likely occurred as a result of reduced rumen motility. Lohuis et al. (1988) reported that administration of LPS in ruminants reduced ruminal contraction intensity and frequency to the extent that rumen motility was depressed for up to 7 h after endotoxin administration. A decrease in rumen motility may also explain the tendency for the decreased DMI observed in LPS-challenged steers. A reduction in DM digested observed in +LPS vs. −LPS steers in spite of slower passage rates and decreased intakes could be explained by an altered rumen environment (such as decreased rumen pH) because of decreased gut motility (and potentially decreased rumination).

Figure 6. Plasma Met concentrations in response to bacterial lipopolysaccharide (LPS) administration (0 h) in growing beef steers. Treatments were a 2 × 2 factorial arrangement of no LPS (−LPS; 100 mL of sterile saline) or a prolonged low dose of LPS (+LPS; 2 µg of LPS × kg of BW/100 mL of sterile saline), and dietary supplementation of 0 or 14 g/d of rumen-protected Met (n = 5 per treatment). Effects for Met were LPS × Met × h (P < 0.01), LPS × Met (P = 0.06), LPS × h (P < 0.01), Met × h (P < 0.01), LPS (P = 0.07), and Met (P < 0.01). Error bars are SEM.

Figure 7. Plasma concentrations of Thr, Leu, Ile, Phe, and Trp in response to bacterial lipopolysaccharide (LPS) administration (0 h) in growing beef steers. Treatments were a 2 × 2 factorial arrangement of no LPS (−LPS; 100 mL of sterile saline) or a prolonged low dose of LPS (+LPS; 2 µg of LPS × kg of BW/100 mL of sterile saline), and dietary supplementation of 0 or 14 g/d of rumen-protected Met (n = 5 per treatment). Error bars are SEM.
Table 3. Dietary intake, digestion, N balance, and ruminal passage rates of growing beef steers in response to Met supplementation and endotoxin (lipopolysaccharide; LPS) challenge

<table>
<thead>
<tr>
<th>Item</th>
<th>−LPS −MET</th>
<th>−LPS +MET</th>
<th>+LPS −MET</th>
<th>+LPS +MET</th>
<th>SEM</th>
<th>LPS × Met</th>
<th>LPS</th>
<th>Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, g/d</td>
<td>3,755</td>
<td>3,474</td>
<td>3,494</td>
<td>2,408</td>
<td>102</td>
<td>0.95</td>
<td>0.13</td>
<td>0.95</td>
</tr>
<tr>
<td>Intake</td>
<td>78.2</td>
<td>73.2</td>
<td>74.2</td>
<td>5.0</td>
<td>0.99</td>
<td>0.37</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Fecal</td>
<td>993</td>
<td>27.8</td>
<td>30.0</td>
<td>1.4</td>
<td>0.38</td>
<td>0.82</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Digested</td>
<td>2,762</td>
<td>45.5</td>
<td>44.3</td>
<td>4.0</td>
<td>0.76</td>
<td>0.24</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>N, g/d</td>
<td>30.8</td>
<td>50.4</td>
<td>47.2</td>
<td>5.8</td>
<td>0.70</td>
<td>0.01</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>3.2</td>
<td>7.0</td>
<td>6.9</td>
<td>1.5</td>
<td>0.87</td>
<td>0.04</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Retained</td>
<td>21.2</td>
<td>−5.0</td>
<td>−2.9</td>
<td>3.0</td>
<td>0.26</td>
<td>&lt;0.01</td>
<td>0.61</td>
<td></td>
</tr>
</tbody>
</table>

Passage, %/h

Cr 4.81 5.29 2.88 3.85 0.30 0.44 <0.01 0.03
Yb 2.49 2.88 1.38 2.08 0.21 0.47 <0.01 0.02

1Treatments were a 2 × 2 factorial arrangement of: 1) no LPS (−LPS; 100 mL of sterile saline) or 2) a prolonged low dose of LPS (+LPS; 2 µg of LPS × kg of BW/100 mL of sterile saline) and dietary supplementation of 1) 0 g/d (−MET) or 2) 14 g/d (+MET) of rumen-protected Met.

2For n = 5.

Figure 8. Plasma concentrations of Ser, Gly, Ala, Asn, Glu, and Orn in response to bacterial lipopolysaccharide (LPS) administration (0 h) in growing beef steers. Treatments were a 2 × 2 factorial arrangement of no LPS (−LPS; 100 mL of sterile saline) or a prolonged low dose of LPS (+LPS; 2 µg of LPS × kg of BW/100 mL of sterile saline), and dietary supplementation of 0 or 14 g/d of rumen-protected Met (n = 5 per treatment). Error bars are SEM.
Administration of LPS increased urinary N excretion by 55% and decreased N retention by 145% for +LPS steers compared with −LPS steers, such that +LPS steers were in negative N balance. Cole et al. (1986) and Orr et al. (1988) reported decreases in N retention and increased urinary N excretion of calves inoculated with IBRV. Orr et al. (1988) attributed these alterations to an increase in N catabolism, as indicated by a reduction in the half-life of $^{15}$N-labeled Gly in IBRV-infected calves. Metabolic demand for N increases during inflammation because AA are utilized for the synthesis of acute-phase proteins, immune cells, and glucogenic precursors (Spurlock, 1997). Reeds and Jahoor (2001) speculated that the increased N excretion observed in humans during sepsis occurred because of imbalances between the supply of AA derived from tissue protein and the AA composition of acute-phase proteins. These imbalances subsequently result in the mobilization of excess tissue protein and AA, which are not utilized for acute-phase protein synthesis. These excess AA may be catabolized for removal of excess N or utilized for the synthesis of glucose precursors. Excess amino-N from these processes likely contributes to the greater plasma urea-N observed in IBRV-infected calves (Orr et al., 1988) and the increase in ammonia released into the urine observed during sepsis (Dejong et al., 2004). Therefore, the increases in plasma urea-N and urinary N excretion observed in +LPS steers likely reflect increased catabolism of N-containing compounds.

**Met Supplementation**

The increased plasma Met concentrations observed in Met-supplemented steers before administration of LPS indicated that the rumen-protected Met effectively delivered absorbable Met. The observed decline in plasma Met concentrations of Met-supplemented steers challenged with LPS suggests that the metabolizable Met provided was either utilized by the immune system or catabolized by the liver. However, the absence of an LPS × Met interaction for N retention implies that LPS-challenged steers did not exhibit increased requirements for Met. It is also possible that Met was not the most limiting AA. Although the basal diet was predicted to supply adequate Met (based on the Cornell Net Carbohydrate and Protein System, version 5.0.40; Dept. of Animal Science, Cornell Univ., Ithaca, NY) under nonstressful conditions, we hypothesized that Met would become limiting under the conditions imposed by LPS. Considering the observed declines in plasma concentrations of other essential AA (Thr, Lys, Leu, Ile, Phe, Trp), it is plausible that one or more AA other than Met may have been limiting or colimiting. Plasma concentrations of Lys, Leu, Ile, and Phe in general were less than the concentrations of these AA previously reported by Löest et al. (2002) and Schroeder et al. (2006a,b). Therefore, a limitation in one or more of these AA in this study may have masked the response to supplemental Met in LPS-challenged steers. In addition, a potentially limited supply of dietary energy (dietary NE $= 0.93$ Mcal/kg) may have attenuated the potential for a response to the additional Met supplied to LPS-challenged steers. If the dietary energy supply was limiting in LPS-challenged steers, then the supplemental Met provided may have been utilized for the synthesis of glucose precursors.

The decreased plasma concentrations of Val observed in steers supplemented with rumen-protected Met is a typical plasma branched-chain AA response in Met-supplemented cattle (Campbell et al., 1997; Waterman et al., 2007), and can be indicative of less AA utilization because of limitations imposed by a Met deficiency. However, no associated responses were observed for other plasma AA, plasma urea-N, or N retention. Therefore, it is plausible that a decrease in plasma Val concentrations was due to competition between Met and the branched-chain AA for absorption. Langer et al. (2000) proposed that interactions among the branched-chain AA and Met exist and that Met may compete with branched-chain AA for uptake into tissues because of a common transport system.

Fecal DM and N excretion were greater in Met-supplemented steers, likely as a result of the increase in solid and liquid passage rates observed in response to Met supplementation. Munn (2004) also observed an increase in liquid passage rate in response to supplementation of rumen-protected Met. The increase in passage rate attributed to rumen-protected Met supplementation in this study may have occurred because of altered microbial fermentation. Microbial protein synthesis, fiber digestion, and in vitro fermentation have been reported to increase in response to dl-Met supplementation (Clark and Petersen, 1988; Patterson and Kung, 1988). It is possible that a portion of the Met from the rumen-protected source used in this study was released into the rumen. Although Met supplementation did not alter N utilization in LPS-challenged steers, serum haptoglobin concentrations were greater in Met-supplemented steers 24 h after LPS infusion, which suggests that the additional Met provided may have been utilized for acute-phase protein synthesis.

**Conclusions**

Providing supplemental dietary Met to growing beef steers exposed to bacterial LPS does not alleviate the effects of LPS on serum hormone profiles or N utilization. However, the declines in plasma concentrations of Met, Lys, Leu, Ile, Phe, Trp, Gly, Ser, and Asn observed in response to LPS suggest that metabolic demand for AA may increase under conditions in which the immune system is stimulated in growing beef cattle.

**LITERATURE CITED**


